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## Short Communication

# Skin sheds as a useful DNA source for lizard conservation

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**Palavras-chave:** extração de DNA, Lacertidae, lagartos, mudas de pele, *Zootoca vivipara.*

It is widely known that the study of the species' genetics is essential for the development of conservation and management strategies. Conservation aims to maintain genetic diversity because it influences the adaptive (Frankel and Soulé 1981) and evolutionary potential of a species (Koljonen *et al.* 2002). Knowledge about genetic diversity facilitates the identification of management units at the species level (Moritz 1994). This is especially important in small and/or isolated populations because they are expected to lose genetic variation (Ouborg *et al.* 2006) and to suffer inbreeding depression (Ellstrand and Elam 1993) over time. However, obtaining suitable samples may be problematic and should aim at minimizing extinction risks (Reed and Frankham 2003, Kramer and Havens 2009).

A variety of reptile species are only known from small/isolated populations. Obtaining DNA from animals in these populations must be as respectful (non-invasive) as possible in order to avoid problems caused by sampling. Non-invasive DNA sampling would be the safest method, especially from small species. Extracting DNA from faeces is tricky because of fast decomposition. In this sense, one potential non-invasive source of DNA, the shed/moult of the skin, has been successfully employed for DNA extraction in alligators (Yan *et al.* 2005) and snakes (Bricker *et al.* 1996, Clark 1998, Fetzner 1999, Dubey *et al.* 2010). Skin sheds can be collected directly from the individuals, or in the field, since they are usually highly visible.

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Moreover, their decomposition is slower than faeces, which would be an additional advantage.

In this work, skin sheds were obtained from the European common lizard, *Zootoca vivipara* (Lichtenstein, 1823) (Lacertidae), during population sampling. To date, almost all DNA studies carried out in lizards have sampled a small piece of the tail, toes, or blood. Thus, the DNA source tested here will provide a new, currently unused, method of non-invasive DNA sampling. We extracted and PCR-amplified DNA from ten individuals from three different populations (two in France and one in Spain). Eight of these individuals were adults (three females and five males) and two juveniles (one male and one female). In the field, we carefully collected samples of shed skin (originating from many scales) from the lizard with tweezers and put them into (dry) paper envelops at room temperature. In the laboratory, we employed skin sheds of one to six scales (approximately 1mm$^2$ per scale) of each individual for DNA extraction using two different methodologies: i) a DNeasy Blood & Tissue Kit (Qiagen; Verlo, Netherlands), and ii) a Chelex-resine based protocol (Estoup *et al*. 1996). The former technique was used 4 month and the latter 11 months after collection. DNA quantification (Table 1) showed successful DNA extraction in 100% of the samples. The commercial kit extracted between 0.4 (ZV9, shed of 5 scales employed) and 3.8 (ZV8, shed of 3 scales) ng/µl of DNA per sample, and the Chelex method between 24.2 (ZV9, shed of 3 scales) and 48.0 (ZV7, shed of 6 scales) ng/µl of DNA per sample. Absorbance assay was used to measure the purity of nucleic acids ($A_{260/280}$ ~1.8 means pure DNA). It ranged from 0.70 in ZV6 to 2.60 in ZV10 when extracting with the commercial kit and from 0.56 in ZV7 to 1.05 in ZV4 when extracting with Chelex. DNA from both extraction methods led to good quality DNA and enough quantities to allow for PCR amplification. In all samples, Chelex extraction led to significantly higher DNA quantity than the commercial kit (paired t-test: $t_9 = 12.7$, $P < 0.001$), even when employing sheds from a lower number of scales (paired t-test: $t_9 = -2.3$, $P = 0.047$). Moreover, skin shed of one single scale was enough to obtain high DNA quantities when using Chelex. DNA purity was significantly better in the case of the commercial kit (paired t-test: $t_9 = -3.1$, $P = 0.014$), and in all but one sample (ZV6). In sum, the commercial kit provided much less but purer DNA, than the Chelex methodology. The number of scales from which sheds were employed for DNA extraction (between 1 and 6) was not significantly correlated with the quantity of extracted DNA, neither when using the commercial kit (Pearson correlation coefficient = -0.584, $N = 10$, $P > 0.05$) nor when using the Chelex protocol (Pearson = -0.581, $N = 10$, $P > 0.05$). The number of scales from which sheds were employed was also not correlated with the purity ($A_{260/280}$; Pearson = 0.315, $N = 10$, $P > 0.05$ for commercial kit; Pearson = -0.300, $N = 10$, $P > 0.05$ for Chelex protocol). There was no significant correlation of DNA quantity and purity among methodologies (quantity: Pearson = -0.150, $N = 10$, $P = 0.679$; purity: Pearson = 0.049, $N = 10$, $P > 0.05$).

After extraction, two mitochondrial genes were amplified by PCR: NADH dehydrogenase 2 gene (ND2, primers MetF6 and AsnR2; Macey *et al.* 1997) and 16S ribosomal RNA gene (16S rRNA, primers 984 and 986; Clary and Wolstenholme 1985). PCR reactions containing: 5 Prime Master Mix (5 Prime; Hamburg, Germany) and 1-10 ng DNA in a total volume of 25µL. PCR thermal cycling conditions were as follows: initial denaturation at 94ºC for 5 min followed by 30 cycles of denaturation at 94ºC for 30 s, annealing at 50ºC (16S rRNA) or 53ºC (ND2) for 30 s, and extension at 72ºC for 90 s, with a final extension step at 72ºC for 10 min. PCR products were visualized in 1.5% agarose gels with 10 mg/ml ethidium bromide. Negative controls were employed to detect contamination and PCR products were purified using Exosap-it for PCR product cleanup (Affymetrix; Santa Clara, CA, USA) before sequencing. Finally, the sequences were visualized and manually edited

**Table 1**. Results of DNA extraction using two different methodologies (commercial kit and Chelex resin) and PCR amplification of *Zootoca vivipara* skin sheds. Acronyms: quantity (ng/μL) and quality ($A_{260/280}$) of the extracted DNA; shed skin originating from *N* scales employed for the DNA extraction (*N*); age (in years; AD: adult ≥ 3 years), sex (M: male; F: female), population (Pop) and country where they were sampled. The accession numbers of the sequence and the most similar GenBank haplotype (last two columns) are listed for ND2 and 16S rRNA sequences.

| | Commercial kit | | | Chelex resin | | | | | | | GenBank | | Most similar haplotype | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample** | **ng/μL** | **$A_{260/280}$** | ***N*** | **ng/μL** | **$A_{260/280}$** | ***N*** | **Age** | **Sex** | **Country** | **Pop** | **ND2** | **16S rRNA** | **ND2** | **16S rRNA** |
| **ZV1** | 1.2 | 1.38 | 3 | 36.7 | 1.03 | 1 | AD | M | Spain | 1 | KR825258 | KR825248 | KF593874 | AY714965 |
| **ZV2** | 1 | 1.39 | 4 | 25.5 | 0.93 | 1 | AD | M | Spain | 1 | KR825258 | KR825251 | KF593874 | GQ142095 |
| **ZV3** | 1.4 | 1.25 | 4 | 34.4 | 0.82 | 4 | 2 | M | Spain | 1 | KR825258 | KR825251 | KF593874 | GQ142095 |
| **ZV4** | 1.6 | 1.35 | 5 | 32.4 | 1.05 | 5 | AD | M | France | 2 | KR825259 | KR825252 | KF593890 | AY714965 |
| **ZV5** | 1.1 | 1.92 | 6 | 30.3 | 1.03 | 1 | AD | F | France | 2 | KR825260 | KR825253 | KF593881 | AY714967 |
| **ZV6** | 1.3 | 0.7 | 4 | 25.6 | 1.03 | 1 | AD | F | France | 3 | KR825260 | KR825251 | KF593881 | GQ142095 |
| **ZV7** | 0.7 | 1.01 | 6 | 48 | 0.56 | 6 | AD | F | France | 3 | KR825260 | KR825250 | KF593881 | GQ142095 |
| **ZV8** | 3.8 | 1.74 | 3 | 27.4 | 0.99 | 3 | AD | M | France | 3 | KR825260 | KR825253 | KF593881 | AY714967 |
| **ZV9** | 0.4 | 1.18 | 5 | 24.2 | 0.99 | 5 | 1 | M | France | 3 | KR825261 | KR825251 | KF593881 | GQ142095 |
| **ZV10** | 0.8 | 2.6 | 6 | 27 | 0.84 | 6 | AD | M | France | 3 | KR825262 | KR825250 | KF593881 | GQ142095 |

using the BioEdit sequence alignment editor software (Hall 1999).

PCR amplification was assessed using agarose gels and all samples produced bands of the expected length for both mitochondrial genes. Amplification was thus independent of sex, age and origin of the population. Sequencing produced 5 different haplotypes in each of the two partially amplified genes and all sequences were submitted to the GenBank public database (Table 1). BLAST analyses (http://blast.ncbi.nlm.nih.gov) confirmed in all cases that PCR products belonged to *Z. vivipara* and the sequences matched best with three previously known haplotypes (haplotype accession numbers of the most similar GenBank sequences are given in Table 1).

In conclusion, skin sheds of lizards are a useful DNA source, in adults and one-year old individuals and even when only tiny quantities of skin shed are available. This method thus constitutes a new, currently unused, non-invasive method of DNA sampling. This method provided good quality DNA allowing amplifying different mitochondrial genes via PCR. More DNA of lower purity was extracted with the Chelex protocol and less DNA of higher purity was extracted with the commercial kit. Furthermore, skin sheds could be sampled without using alcohol and tubes (reduced equipment costs and reduced weight). The applied methodology is less painful for the animals and requires very little storage space. Sampling of skin sheds thus constitutes an alternative to the frequently employed tail tissue sampling method, which is commonly used in small lacertids.

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